IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Nicholas J. Deacon *et al.*	**Examiner:**	J. Parkin
Serial No.:	08/388,353	**Art Unit:**	1813
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For:	NON-PATHOGENIC STRAINS OF HIV		

Assistant Commissioner for Patents
Washington, DC 20231

DECLARATION PURSUANT TO 37 C.F.R. §1.132

I, Nicholas J. Deacon, declare as follows:

1. I am a citizen of Australia residing at 51 Elliot Avenue, Balwyn, State of Victoria, Australia.

2. I received a Bachelor of Science Degree in Biochemistry from University of London in 1973 and was awarded a Ph.D. in Biochemistry from University of London in 1976.

3. I have held a number of different positions since 1976 which are set forth in my curriculum vitae provided herewith as Exhibit I.

4. I am a named co-inventor of the subject matter in the above-identified application, filed on February 14, 1995, as U.S. Patent Application Serial No. 08/388,353 (hereinafter referred to as the "APPLICATION") and am familiar with the contents therein.

5. The invention of the present APPLICATION is directed, inter alia, to isolated non-pathogenic strains of HIV-1. The subject non-pathogenic strains all comprise a common deletion which is responsible for non-pathogenicity. In particular, all of the isolated non-pathogenic strains carry a deletion corresponding to nucleotides 9281-9437 using the nucleotide numbering of HIV-1 wild type strain NL4-3. The deletion corresponds to amino acids

166-206 of the *nef* gene and includes the U3 portion of the LTR.

6. The subject non-pathogenic strains of HIV-1 were discovered during routine monitoring of data recorded by the New South Wales (NSW) Red Cross Blood Transfusion Service. The NSW Red Cross Blood Transfusion Service established a "Look Back Unit" to monitor persons inadvertently infected with HIV-1 as a result of treatment with blood products e.g., blood transfusion. Out of 130 infected persons, two had not progressed to AIDS even though they had been infected for six years. These individuals were especially interesting since persons infected with HIV-1 *via* transfusion normally progress to full blown AIDS more rapidly than those individuals infected with HIV-1 *via* sexual transmission. For example, of the 122 non-pediatric, transfusion transmitted cases registered in the NSW, the mean time to progression to AIDS was between 6 and 7 years.

7. By the year 1989 it was clear that the two identified individuals (discussed in paragraph 6 *supra)* were still not showing signs of disease progression. Importantly, investigation showed that they had received blood from the same individual donor (D36) who also appeared to be healthy with no sign of disease progression. A subsequent search of the register revealed a total of seven recipients of Blood from D36, all of whom were HIV-1 positive but with no signs of progression to AIDS or HIV-associated illness. These data are as reported in J. Learmont *et al.*, (1992) Lancet 340 :863, attached hereto as Exhibit 2, the seven asymptomatic individuals have been termed the "Sydney Bloodbank Cohort" or "SBBC".

8. All members of the SBBC, including the donor, had stable, normal to (>500 cells/μl blood) CD4+lymphocyte counts and very low levels of HIV-1 provirus (≤ 400 copies/10^5 $CD4^+$ peripheral blood mononuclear cells). These data are as reported in J. Learmont et al. 1992 and Deacon, N.J, *et al.*, (1995) *Science 270:* 988, attached hereto as Exhibits 2 and 3 respectively. Plasma virus load was similarly low (≤ 5600 copies/ml). The members of the cohort are not related and do not share histocompatibility antigen haplotypes as reported in J. Learmont, *et al.*, (1995) *AID Res. Hum. Retroviruses 11:* 1, attached hereto as Exhibit 4. Thus, there appears to be no common host factor to explain the lack of progression to AIDS in the SBBC members. Records indicate that the donor was infected via sexual transmission some time

between December 1980 and February 1981. The only risk factor for the cohort members was blood transfusions, given for the usual varied reasons such as surgery and non-surgery associated bleeds. Table 1 of Deacon *et al.*, 1995, attached herewith as Exhibit 3, is a compilation of transfusion and clinical information for the cohort members.

9. Since there did not appear to be a common host factor to explain the lack of progression to AIDS in the SBBC members, the possibility of a viral factor was investigated. HIV-1 was successfully isolated from one member of the SBBC, recipient C18. Analysis of products of polymerase chain reaction (PCR) amplification of the HIV-1 *nef* gene and long terminal repeat (LTR) regions from C18 showed that they were smaller than the PCR products from the wild-type pathogenic strain HIV-1 NL43 and from clinical isolates of HIV-1 from persons who had progressed to AIDS. Further analysis showed that the nef/LTR region of HIV-1 from the donor D36 and recipients C18, C49, C54, C64, C98 and C124 was smaller than the nef/LTR region of wildtype pathogenic virus, these results are reported in Deacon, N. J. *et al.*, 1995, attached hereto as Exhibit 3.

10. As described in *Retroviruses*, Coffin, J.M., Hughes, S. H. and Varmus, H.E., Editors Cold Spring Harbour, Laboratory Press, New York, USA, 1997; pages 33-36, attached hereto as Exhibit 5, the *nef* gene is situated close to the 3'-end of the HIV-1 genome where its 3'- half (encoding the Nef protein C-terminal region) overlaps the sequences of the LTR and is termed the *nef*/LTR region. The 5'-half of the *nef* gene encodes the N-terminal region of the *Nef* protein and is termed the *nef*-alone region. The mechanism of replication of HIV-1 ensures that the sequence of the LTR at the 5'-end of the HIV-1 genome is the same as that of the 3'-LTR. Deletions, therefore in the 3'-LTR will be observed in the 5'-LTR. The 5'-LTR includes sequences important in the control of HIV-1 gene expression.

11. DNA sequence analysis of the region amplified by PCR of HIV-1 from members of the SBBC demonstrated that sequence was deleted from the *nef*-alone region and from the *nef*/LTR overlap region as indicated in Table 1 of N. J. Deacon *et al.*, 1995 (Exhibit 3). The size and location of the deletions in HIV-1 from the different SBBC members were related but not exactly the same. However, all had a similar 3' -most deletion extending downstream from the

equivalent of nucleotide 9281 to nucleotide 9438 of the HIV-1_{NL43} sequence, in the *nef*/LTR overlap region. This common deleted region includes LTR binding sites for the transcription factors NF-AT, USF, LEF-1 and the 5' NFkB binding site of clade B HIV-1 strains. Partly replacing the deleted sequences were rearranged duplications of the downstream 3'-NFkB-Sp1 site sequences. As reported in Deacon *et al.*, 1995 (Exhibit 3), similar direct analysis of HIV-1 provirus in SBBC member peripheral blood mononuclear cells (PBMC) confirmed the data found for cultured SBBC HIV-1 isolates. All members of the SBBC have HIV-1 with the common 3'-most *nef*/LTR overlap region deletion. None of the members has been shown to have any wildtype, non-deleted HIV-1.

12. The possibility of deletions elsewhere in the HIV-1 genome was investigated by the determination of the full length genomic sequence of uncloned and both biologically and molecularly cloned HIV-1 from recipient C18 as well as of uncloned material from D36, C54 and C98. See Deacon *et al.*, 1995 (Exhibit 3) and R. Oelrichs, *et al.*, (1998) in press in *AIDS*, attached hereto as Exhibit 6. Among C18, D36, C54 and C98, the only significant differences from the wild-type pathogenic strains of HIV-1 are the deletions in the 5'-and 3'-LTR regions, the *nef*-alone region and the duplication of NFkB-Sp1 site sequences.

13. Three members of the SBBC have died: C18, C83, and C124. Only with C83 has there been any suggestion of a link with HIV-1 infection/AIDS, as her final illness included Pneumocystis carinii pneumonia (PCP). C83 had the autoimmune disease systemic lupus erythematosis (SLE) and it was during a SLE associated bleed in December 1982 that C83 received the transfusion of D36 blood. Her therapy included immunosuppressive doses of prednisone, azathioprine and cyclophosphamide and she was lymphopenic lymphocyte count 300 x 10^6 cells/L) on her final admission to hospital when PCP was diagnosed. C83 was found to be seropositive for HIV-1 just 2 weeks before her death in April 1987. A sample of DNA extracted from PBMC (of unknown CD4+ lymphocyte concentration) taken about 2 years before death has been analysed by DNA sequencing of products of quadruple nested PCR amplification of the HIV-1 *nef*/LTR region. This demonstrated the deletion of sequence including the common 3'-most deletion region. In the absence of suitable samples from that time it is now impossible to distinguish between death due to SLE plus PCP due to immunosuppressive therapy, and death

related to HIV-1. Two years prior to death however, the level of HIV-1 provirus in PBMC DNA sample was exceedingly low and the HIV-1 was of the SBBC *nef*/LTR-deleted strain.

14. During the period December 1980 to July 1984, the donor D36 made 16 donations of his blood to the NSW Blood Transfusion Service. Seven of these led to the infection of the recipients already noted. Continued searching of Blood Transfusion Service and hospital records resulted in the identification of an eighth recipient, C135. This recipient was the earliest infected of the SBBC (Table 1 of N.J. Deacon *et al.*, attached hereto as Exhibit 3) and was found to be weakly seropositive to HIV-1 with an indeterminant western blot. C135 had an undetectable virus load (<200 RNA genome copies/ml plasma), a normal $CD4^+$ lymphocyte level and no signs of progression to AIDS. C135, alone of the SBBC is heterozygous for the Δ32 allele of the CKR5 chemokine receptor. Analysis by DNA sequencing of triple nested PCR amplified HIV-1 *nef*/LTR region demonstrated the presence of *nef*-alone and the *nef*/LTR overlap region 3'-most common deletions confirming it as a SBBC strain of HIV-1. No wild-type non-deleted HIV-1 was observed.

15. Reports of non-pathogenic SIV and HIV-1 infection, similar to the SBBC, have been made in the literature. As reported in H.W. Kestler III *et al.*, (1991) Cell 65:651, attached hereto as Exhibit 7, inoculation of macaques with molecularly cloned SIVmac239 with a large deletion (about 183 bp) of the *nef*-alone region sequence (SIVmac239Δnef) resulted in infection of the macaques with a low level of virus production and no progression to disease. Following infection, additional deletions in the *nef* gene, including the *nef*/LTR overlap region, occurred. The only other reported case involved a hemophiliac infected for 10 years with HIV-1 having a deletion in the *nef*-alone region. See F. Kirchhoff, *et al.*, (1995) *N. Engl. J. Med. 332:* 228, attached hereto as Exhibit 8. Low provirus load, a normal CD4+ lymphocyte concentration and no progression to AIDS were noted. Again, additional deletions in the *nef*-alone and *nef*/LTR regions were seen with increasing time after infection. No analysis of the HIV-1 proviral sequence outside of the *nef* gene and LTR regions was reported.

16. In summary, the SSBC is the first recorded case of transmission of a replication competent strain of HIV-1 from a common donor to a group of recipients who became HIV-1

positive but have not progressed to disease. As the only obvious defect in the genome of the donor strain D36 is the deletion of sequences from the *nef* gene and LTR regions, this finding indicates that the deletion is the basis of the lack of pathogenicity of the virus.

17. The present specification discloses that a mutation in the LTR region of a subject non-pathogenic HIV-1 isolate occurs 5' of the Sp1 sites. The first Sp1 site in the LTR occurs just after nucleotide 9438. Page 25 of the subject specification indicates that in SEQ ID NO:614 (D36), nucleotide 9281 begins after the fourth T on line 33 of SEQ ID NO:614. The sequence prior to 9281 is ATTGTT. The next nucleotide would be 9281 and is the beginning of the deletion. The deletion extends to nucleotide 9437 which can be seen after the fourth G on line 34 of SEQ ID NO:614. The nucleotide sequence in between 9280 and 9438 is the result of "fill in" and duplication which is amply described in the specification. Thus, the existence of the deletion from 9281-9437 is clear to a skilled artisan by comparing SEQ ID NO:614 with the nucleotide sequence of the corresponding region in wild type HIV-1 strain NL4-3.

18. Similarly, SEQ ID NO:615 (C18) on page 26 of the subject specification shows the same deletion, i.e., nucleotides 9281-9437. The beginning of the nucleotide deletion, i.e., nucleotide 9281 occurs after the fourth T on line 21. The deletion ends after the eighth C on line 22. The existence of deletion 9281-9437 is thus clear to a skilled artisan by comparing SEQ ID NO:615 with the wild type sequence of NL4-3. Figure 5 of the present specification also indicates mutations beyond nucleotides 9437 in the NFkB region of the LTR prior to the Sp1 sites.

19. Isolate C18 has been completely sequenced and evidences no differences from the wild type HIV strain except for the mutation corresponding to amino acids 166-206 of the *nef* gene and extending into the U3 region of the LTR. Full length sequences for clones C54, C98 and D36 have been determined with consistent results. Those individuals infected with the subject HIV-1 strains do not exhibit AIDS symptoms over time; indeed the individuals exhibit normal CD4+ levels. Thus, while non-pathogenicity can be effected in many ways, the deletions of the present invention offer a consistent basis of attenuation which is clinically measurable on live individuals.

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20. I further declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the APPLICATION or any patent issued thereon.

Dated: 23 · 4 · 98

Nicholas J. Deacon